simulation can provide a detailed microscopic picture of lipid behavior in the ripple phase. In the first simulations of their kind, we study stacks of rippled bilayers to provide a more realistic molecular perspective that can be directly compared to X-ray diffraction and AFM experiments. We find that saddle-splay and tilt are key order parameters that characterize the long-range order observed in ripple phases. In addition, we elucidate the effects of lipid length, head group interaction, and bilayer hydration on ripple structure. The impact of lipid orientations in the ripple phase to biological processes such as membrane fusion is discussed.

1121-Plat

Influence of Detergent Properties on the Solubilization and Function of Membrane Proteins

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Detergent micelles are often used as a bilayer mimic for membrane proteins for functional assays and high-resolution structural studies, but selecting the appropriate detergent for the protein requires extensive time and resources. The long-term goal of this research is to rationally select a membrane mimic for a given membrane protein that stabilizes fold and native function based on matching physical properties of the detergent and protein. To determine the important micelle and membrane properties, lipoprotein signal peptidase A (LspA) from Chlamydia trachomatis is used as a model system. To screen detergents that support the native function, LspA was purified in over 30 detergent micelles with varying physical properties. LspA is soluble in zwitterionic detergents and solubility remains independent of detergent alkyl chain length, solubilizing in detergents with wide range of hydrophobic radius, 27-37Å. A detergent may maintain protein solubility without maintaining function or fold; therefore, the function of LspA in these detergents is currently being studied. To assess the activity of native membrane and solubilized LspA, a signal peptide containing the LspA cleavage consensus sequence and donor-quencher pair on each termini was designed. Preliminary HPLC and fluorescence data demonstrate a cleavage of the signal peptide upon addition of LspA in the native membrane and a comparison to different protein-detergent complexes will be presented. The results presented will identify solubilizing detergents, monitor enzymatic activity, and ultimately correlate the physical properties of the solubilizing detergent and protein structure and function.

1122-Plat

Quantitative Imaging of the Electrostatic Field of a Transmembrane Protein at Subnanometer Resolution by the use of Atomic Force Microscopy

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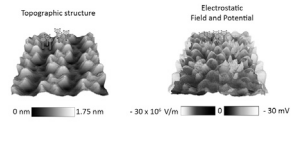
Elucidating the mechanisms by which proteins translocate small molecules and ions through transmembrane pores and channels is of great interest in biology, medicine and nanotechnology. These translocation mechanisms are mainly based on electrostatic interactions. However, the characterization of pore forming proteins in their native state lacks suitable methods that are capable of high-resolution imaging (≈1 nm) while simultaneously mapping physical and chemical properties. Here we report how force-distance (FD) curve based atomic force microscopy (AFM) imaging can be applied to image the native pore forming outer membrane protein F (OmpF) at sub-nanometer resolution and to quantify the electrostatic field and potential generated by the transmembrane pore. We further observe the electrostatic field and potential of the OmpF pore switching 'on' and 'off' in dependence of the electrolyte concentration. Because electrostatic field and potential select for charged molecules and ions and guide them to the transmembrane pore the insights are of fundamental importance to understand the pore function. These experimental results establish FD-based AFM as unique tool to image biological systems to sub-nanometer resolution and to quantify their electrostatic properties.



1123-Plat

Continuous Flow AFM Imaging Reveals Fluidity and Time Dependent Interactions of Antimicrobial Dendrimer with Model Lipid Membranes

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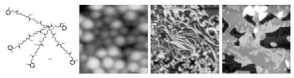
The alarming increase in multi-drug resistant bacteria worldwide is a major threat to modern life and there is thus an urgent need to address this issue by novel approaches in order to improve and tailor alternative drug formulations in the future. The nanoparticle studied here is a newly synthesized antimicrobial peptide-based dendrimer that acts by disrupting cell surfaces in a non-specific manner, thus rendering it less susceptible to resistance-forming mechanisms in bacteria.
The effects of bilayer fluidity and presence of domains have been studied using neutron reflection and an atomic force microscope setup optimized for continuous flow imaging, developed specifically for investigating interactions with fast kinetics.
Using these approaches we observed a requirement for membrane fluidity for dendrimers to induce high curvature and solubilize the membrane in a detergent-like manner. Domain coexistence led to a sequence of events initiated by the formation of a dense thread-like network and followed by membrane solubilisation via spherical aggregates from bilayer edges.
In contrast, for gel-phase membranes, the antimicrobial dendrimers adsorbed and caused areas of locally depressed regions - a mechanism resembling membrane interdigitation or membrane thinning.



1124-Plat

Exploring Continuum Models of Ion and Peptide Interactions with the Membrane

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Experimental and computational studies have shown that cellular membranes deform to stabilize the inclusion of transmembrane (TM) proteins harboring charge. Recent analysis suggests that membrane bending helps to expose charged and polar residues to the aqueous environment and polar head groups. We previously used elasticity theory to identify membrane distortions that minimize the insertion of charged TM peptides into the membrane. Here, we extend our work to consider the energetics of ion and small peptide penetration into the membrane. First, we show that our continuum method accurately reproduces energy profiles and membrane shapes generated from molecular simulations of bare ion permeation at a fraction of the computational cost. Next, we demonstrate that the apparent linear dependence of bare ion insertion energy on membrane thickness arises primarily from the elastic property of the membrane. Moreover, the continuum model readily provides free energy decompositions, still an obstacle for traditional molecular dynamics (MD). Finally, we show that the energetics of membrane deformation strongly depend on membrane patch size both for ions and peptides. This work therefore presents a novel, computationally efficient method for simulating the dynamics of small molecule and peptide interactions with the membrane bilayer.

1125-Plat

Histones and DNA Compete for Binding Phosphoinositides in Bilayers

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Recent discoveries on the presence and location of phosphoinositides in the eukaryotic cell nucleus prompted us to study the putative interaction of histones with these lipids in model membranes (liposomes). Turbidimetric studies revealed that a variety of histones and histone combinations (H1, H2AH2B, H3H4, octamers) caused a dose-dependent aggregation of phosphatidylcholine vesicles (LUV or SUV) containing negatively-charged phospholipids. 5 mol % PIP was enough to cause extensive aggregation under our conditions, while with PI at least 20 mol % was necessary to obtain a similar effect. Histone binding to GUV and vesicle aggregation was visualized by confocal microscopy. Histone did not cause vesicle aggregation in the presence of DNA, and the latter was able to disassemble the histone-vesicle aggregates. At DNA/H1 weight ratios 0.1-0.5 DNA- and PIP-bound H1 appear to coexist. Isothermal calorimetry studies revealed that the PIP-H1 association constant was one order of magnitude higher than that of PI-H1, but several orders of magnitude lower than that of H1-DNA. The results suggest that, in the cell nucleus, a complex interplay of histones, DNA and phosphoinositides may be taking place. The model system described here could help in analyzing these interactions.